# A screening approach to identify the effector AvrPc2 in *Puccinia coronata*

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## Introduction

- *Puccinia coronata* f. sp. *avenae* (Pca) is a pathogenic fungus that causes crown rust, the most important foliar disease in cultivated oat (*Avena sativa*) (Figure 1).
- Pca displays high genetic variability because it is capable of undergoing sexual reproduction in buckthorn (Figure 2).
- Such genetic diversity among populations of Pca is troublesome because it enables the appearance of races with new virulence traits.
- New races of Pca allow infection of previously resistant oat varieties.
- To date, we know that virulence traits are the result of the pathogen's secretion of one or several molecules known as effectors. Effectors allow the fungus to grow in the plant and suppress defenses.
- One strategy to prevent disease is using plant varieties with resistance (R) genes that allow recognition of effectors. Thus, the plant mounts a defense response against pathogens.
- In 1927, an oat variety (Victoria) resistant to crown rust was introduced to the United States. The success of Victoria oats against crown rust was attributed to the presence of a resistance dominant gene, *Pc2*.
- However, in the following years a new disease called Victoria Blight, caused by the fungus *Cochliobolus victoriae* (Cv) emerged and selectively destroyed all Victoria oats.
- The virulence of Cv in Victoria oats was associated to the production of a host-selective toxin, victorin (Figure 3).
- Classical genetic approaches suggest that susceptibility to Cv and resistance against Pca is given by the same gene, *Pc2*.
- The effector (AvrPc2) that is recognized by the R protein coded by *Pc2* is unknown, which poses an interesting biological question, does victorin and AvrPc2 in Pca share structural commonalities?
- Based on previous evidence, a hypothetical model (Figure 4) was developed to explain the mechanism by which *P. coronata* is able to suppress basal defenses in the plant (Lorang et al. 2012).
  - Victorin binds to a thioredoxin (TRX) preventing the activation of NPR1.
  - *P. coronata* may use AvrPc2 to prevent activation of defenses.
  - AvrPc2 possibly inactivates TRX, thus inhibiting the function of NPR1, the master regulator of basal defenses.
- Oat is an important crop in MN and other regions of the country (Figure 5) and understanding virulence mechanism is important to manage crown rust disease.


Figure 1. Typical symptoms of infection by *P. coronata* in oat.


Figure 2. Typical symptoms of infection by *P. coronata* in buckthorn.

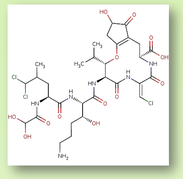


Figure 3. Victorin is a chlorinated cyclic pentapeptide that is produced by the pathogenic fungus C. *victoriae*.

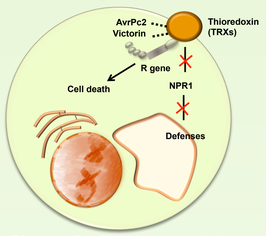



Figure 4. Working model for recognition of AvrPc2

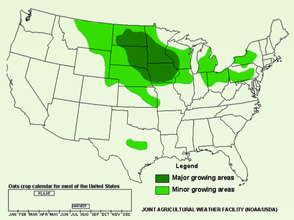



Figure 5. Main oat producing areas in USA

## Goal of this project and rationale

- To set the foundation to identify and clone *AvrPc2* in various isolates of *P. coronata* (Pca). Thus, we can understand how it works in the plant cell.
- To attain this goal we must first recapitulate the original interaction between the AvrPc2-containing Pca race and *Pc2*-containing (resistance gene) oats.

## Methodology

- Utilize the USDA-ARS Cereal Disease Laboratory's collection of Pca to locate isolates of interest

### I. Work Pipeline to test interaction between Pca isolates and oat variaties

Retrieve Pca races 203, 202, and 293 from Liq. $N_2$ collection

Increase inoculum using the susceptible oat variety Marvelous

Confirm physiological race by phenotyping a set of oat differentials

Amplify inoculum in same susceptible oat variety Marvelous

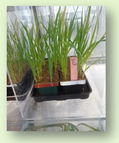
Record symptom development in varieties Victoria and Gary to examine the Pc2-Avr2 interaction

Microscopic examination of infected leaf tissue

Genomic DNA extraction

### II. Protocol to inoculate oat plants with Pca

- Plants were planted and allowed to grow 10 days before inoculation



Use freshly collected spores of Pca
Or
Use spores that are stored at -80°C (with a heat shock at 43°C)

Resuspend spores in soltrol oil

Spray spore solution over plants

Plants are kept in a mist chamber overnight

Plants are transferred to a greenhouse

Symptoms are examined 12 days after inoculation

## Results

- Isolate Pca race 203 was located in the USDA-ARS collection and tested using our work pipeline (see methods).
- Pca race 203 induced cell death in oat varieties Gary and Victoria; whereas in the susceptible variety Marvelous this type of reaction was absent (Figures 6 and 7).
- Plant resistance was also manifested as a decreased number of pustules (fungal colonies) (Figure 6).


Figure 6. Phenotypic analysis of inoculated plants. Induced cell death in Pc2-containing oat varieties Gary and Victoria. Variety Marvelous is a used as a positive control as it is an universal susceptible genotype to oat crown rust.

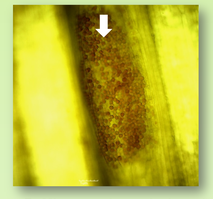


Figure 7. Microscopic analysis of infected tissue. Cell death in Pc2-containing in oat variety Victoria is manifested as a browning of the tissue that surrounds the fungal colony. Arrow shows sporulation of Pca

- Approximately 3 g of spores were produced to continue work with this isolate.
- Calculation of germination rate of our stock isolate was ~70% (Figure 8).

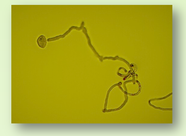


Figure 8. Example of a germinated Pca spore in a 1% agar plate.

- Race designation of isolate Pca 203 was confirmed by using a standard set of differentials (Figure 9); however, we determined that there was a contaminant and isolate Pca race 203 is not a pure isolate.
- To solve this problem, isolation of several single pustule-derived cultures is on-going.

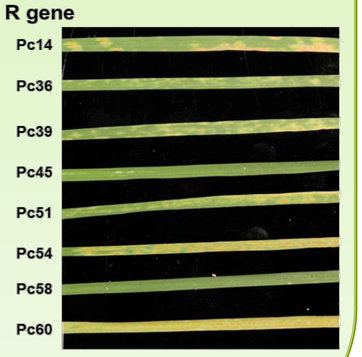



Figure 9. Example of phenotypes in a selected set of oat differentials.

## Conclusions and future work

- The interaction of AvrPc2 and the resistance gene Pc2 was recapitulated. Nonetheless, other Pca isolates predicted to encode AvrPc2 need to be screened to assist with the cloning of AvrPc2.
- Cloning AvrPc2 will help us to understand how the effectors work in the plant cell.
- We are also gearing up to sequence the genome of a representative isolate of Pca.

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